Opiates In Urine-Oxime TMS Procedure For GC OR GC/MS Confirmations Using: 200 mg Clean Screen® Extraction Column

UCT Part Numbers

ZSDAU020 Clean Screen® DAU 10 mL, 200 mg sorbent Without Tips	Or	**ZCDAU020** Clean Screen® DAU 10 mL, 200 mg sorbent With CLEAN-THRU® Tips

Procedure:

1. Prepare Sample Acid Hydrolysis of Glucuronides

a) To 2 mL of urine add internal standard(s)* and 400 µL concentrated HCl.
b) Add 200 µL 10% Hydroxylamine solution in DI H_2O.
c) Mix/vortex.
d) Heat to 90°C for 40 min in a heating block, or an autoclave for 15 minutes on a liquid cycle.
e) Cool before proceeding.
f) Centrifuge for 10 minutes at 2000 rpm and discard pellet.
g) Add 500 µL 50% Ammonium Hydroxide. Mix/vortex.
h) Adjust sample pH 5 - 6 by drop wise addition with 50% Ammonium Hydroxide.

2. Prepare Enzyme Hydrolysis of Glucuronides

a) To 2 mL of urine add internal standard(s)* and enzyme preparation in buffer
b) Mix/vortex.
c) Heat to 60 °C for sufficient time in a heating block (depends on analytes and enzyme)
d) Add 200 µL 10% Hydroxylamine solution.
e) Heat to 60°C for 30 min in a heating block.
f) Adjust pH to 5 - 6
g) Centrifuge for 10 minutes at 2000 rpm and discard pellet.

3. Condition Clean Screen® Extraction Column

a) 1 x 3 mL CH_3OH.
b) 1 x 3 mL D.I. H_2O.
c) 1 x 3 mL 100 mM phosphate buffer (pH 6.0).

Note: Aspirate at < 3 inches Hg to prevent sorbent drying.

4. Apply Sample

a) Load at 1 to 2 mL/minute.

5. Wash Column

a) 1 x 3 mL D.I. H_2O.
b) 1 x 3 mL 100 mM acetate buffer (pH 4.5).
c) 1 x 3 mL CH_3OH.
d) Dry column (5 minutes at > 10 inches Hg).

6. Elute Opiates

a) 1 x 3 mL CH_2Cl_2/IPA/NH_4OH (76:20:4)
b) Collect eluate at 1 to 2 mL/minute.

Note: Prepare elution solvent daily.

c) Add IPA/NH_4OH, mix, then add CH_2Cl_2 (pH 11-12).

7. Dry Eluate

a) Evaporate to dryness at < 40°C.

8. Derivatize

a) Add 100 µL ethyl acetate and 100 µL BSTFA (with 1% TMCS)***.
b) Overlay with N_2 and cap. Mix/vortex.
c) React 45 minutes at 70°C. in a heat block.
d) Remove from heat source to cool.

Note: Do not evaporate BSTFA solution.

9. Quantitate

a) Inject 1 to 2 µL onto gas chromatograph. For MSD monitor the following ions:

FORENSICS

UCT

Compound	Quant Ion**	Secondary	Tertiary	Cerilliant #
Meperidine-D_4	251	222	250	M-036
Meperidine	247	218	246	M-035
Normeperidine-D_4 TMS*	308	280	309	N-020
Normeperidine TMS*	305	276	304	N-017
Tramadol TMS	335	245	290	T-027
O-Desmethyltramadol TMS	393	378	303	T-035
N-Desmethyltramadol TMS	393	378	116	D-023
Pentazocine TMS	357	342	289	P-039
Codeine-D_3 TMS*	374	359	346	C-039
Codeine-D_6 TMS*	377	349	316	C-040
Codeine TMS	371	356	343	C-006
Norcodeine TMS	429	414	356	N-005
Dihydrocodeine TMS	373	315	358	D-019
Morphine-D_3 TMS*	432	417	404	M-099
Morphine-D_6 TMS*	435	420	404	M-085
Morphine TMS	429	414	401	M-005
Normorphine TMS	487	472	414	N-006
Diacetylmorphine	369	327	268	H-038
Hydrocodone Oxime-D_3 TMS	389	300	374	H-008
Hydrocodone Oxime-D_6 TMS	392	303	377	H-047
Hydrocodone Oxime TMS	386	297	371	H-003
Hydromorphone Oxime-D_3 TMS	447	432	358	H-010
Hydromorphone Oxime TMS	444	429	355	H-004
Oxycodone Oxime-D_3 TMS	477	462	420	O-006
Oxycodone Oxime-D_6 TMS	480	465	420	O-008
Oxycodone Oxime TMS	474	459	417	O-002
Oxymorphone Oxime-D_3 TMS	535	520	290	O-019
Oxymorphone Oxime TMS	532	517	287	O-004

*Suggested internal standards for GC/MS: D_4-Meperidine, D_4-Normeperidine, D_3-Codeine, D_3-Morphine D_6-Hydrocodone D_6-Oxycodone

*Suggest trying D_6-Codeine, and D_6-Morphine for lowest LOD/LOQ
*** Part # SBSTFA-1-1, 10, 25, 100

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 • 800.385.3153 • 215.781.9255
www.unitedchem.com Email: methods@unitedchem.com

FORENSICS

UCT